Charge heterogeneity analysis of monoclonal antibodies using CESI-MS

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ABSTRACT

CESI-MS was employed to simplify the characterization of monoclonal antibodies (mAbs). The charge heterogeneity, potential critical quality attributes (CQAs),and glycosylation profiles, can be determined in a single intact analysis. Further, the same CESI-MS platform can be employed to localize and confirm the identity of the charged variants by subunit analysis and peptide mapping.

INTRODUCTION

Therapeutic mAbs can exhibit significant micro-heterogeneity due to the numerous post-translational modifications (PTMs), sequence variants, and degradation products that occur during production and storage. CQAs, such as deamidation, oxidation, and glycosylation can affect efficacy, bioavailability, and biosafety. Therefore, the comprehensive characterization of therapeutic mAbs is essential for both product development and process control.

Integrating CE and electrospray ionization (ESI) into one dynamic process (CESI) simplifies coupling CE with MS[1]. In this work we demonstrate how CESI-MS can be used for the rapid, comprehensive characterization of mAb charge variants at the intact, subunit and peptide level.

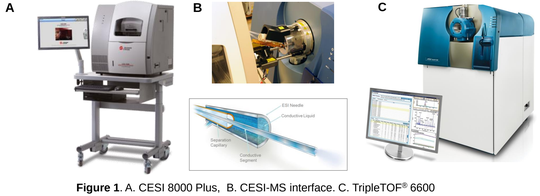


Figure 1. A. CESI 8000 Plus, B. CESI-MS interface. C. TripleTOF® 6600

MATERIALS AND METHODS

Sample Preparation: Trastuzumab was diluted in the leading electrolyte (LE). Subunits were achieved using 20 mM dithiothreitol (DTT) at 80ºC. Rapigest, DTT and iodoacetamide (IAM) were employed to denature, reduce and alkylate for peptide mapping. An overnight digestion with trypsin was achieved at 37ºC. The LE of the digested sample was 133 mM ammonium acetate (pH 4).

cIEF: was performed using the SCIEX cIEF protocol on the SCIEX CESI 8000 Plus system.

CESI-MS: All charge variant experiments were carried out on a SCIEX CESI 8000 Plus system. CE separations of the intact and subunit charge variants were achieved using capillaries with a positive polyethyleneimine (PEI)[2] surface coating while peptide mapping was achieved using a bare fused silica capillary. MS compatible background electrolytes (BGEs) containing acetic acid were used.

MS Conditions: A SCIEX TripleTOF® 6600 system equipped with a NanoSpray® III source and CESI adapter was used for all assays. For intact and subunit analysis the MS mass range was 600 – 4000 m/z in full scan mode. Peptide mapping used 100-2000 m/z in information dependent acquisition (IDA) mode with a 100 msec TOF MS survey scan, a 50 msec IDA, and a rolling collision energy.

Data Analysis: High resolution MS spectra were analyzed using SCIEX PeakView® and BioPharmaView™ software as well as Protein Metrics Intact Mass software.

RESULTS

1. *Intact Analysis of Trastuzumab*

Post-translational and storage-induced modifications often alter the net charge of mAbs consequently changing the electrophoretic mobility. This can be exploited to separate intact mAb charge variants. Trastuzumab was assessed in this study as it is well characterized and its CQAs are well understood. The dominant charge variants of Trastuzumab are deamidation and aspartate isomerization.

cIEF and cation exchange (CEX) chromatography are conventional approaches to characterize charge variants, but the buffers are not compatible with MS. Therefore, LC-MS is still required to identify CQAs, obtain intact masses and determine glycosylation profiles.

CESI-MS provides charge variant profiles, intact masses and glycosylation profiles in a single assay. Figure 2 reveals the CESI-MS separation of Trastuzumab, the insert depicts the cIEF of Trastuzumab and highlights the profile similarities between the two assays.

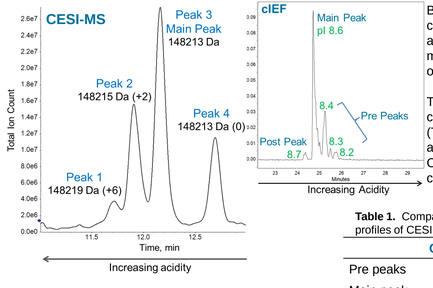


Figure 2. CESI-MS peak profile of Trastuzumab. Insert is the cIEF of Trastuzumab.

Both CESI-MS and cIEF reveal charge variants of increasing acidity. The CESI-MS profile is a mirror image of cIEF, as it is operated in reverse polarity.

The CESI-MS peak profile was compared with cIEF and CEX (Table 1). Agreement between assays highlights the suitability of CESI–MS for charged variant mAb characterization.

Table 1. Comparison between Trastuzumab peak profiles of CESI-MS, cIEF, and CEX

	CESI (%)	cIEF (%)	[4]CEX (%)
Pre peaks	26.7	34.2	27.8
Main peak	58.3	60.4	57.7
Post peaks	15.0	4.0	14.5

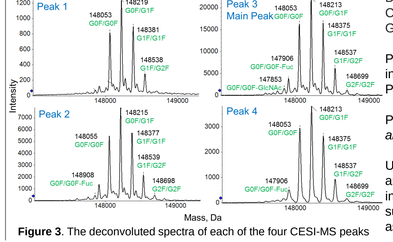


Figure 3. The deconvoluted spectra of each of the four CESI-MS peaks

Deconvoluted spectra identifies potential CQAs and glycosylation profiles (Figure 3). G0F/G1F is the dominant glycoform.

Peak 2 is +2 Da greater than the Main Peak indicating two possible deamidations. While Peak 1 may suggest multiple deamidations.

Peak 4 is a 0 Da shift, using CEX, Harris *et al.* attributed this to a D → IsoD conversion.[3]

Unequivocal assignment of N deamidation and D isomerization (ΔM = + 1 & 0 Da) on intact mAbs is challenging. Analysis of subunit and peptides can confirm assignment and permit site localization.

2. *Subunit analysis*

The analysis of antibody subunits enables the localization of modifications to a specific fragment. Trastuzumab was reduced to its heavy (HC) and light chain (LC) subunits with DTT. Reduced samples were analyzed directly with no additional sample prep.

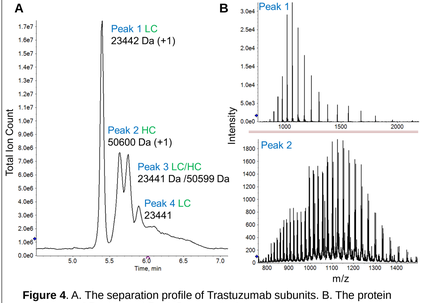


Figure 4. A. The separation profile of Trastuzumab subunits. B. The protein envelopes of Peak 1 and Peak 2 respectively.

The separation profile reveals four peaks (Figure 4A). The protein envelope of Peak 1 is indicative of a LC, while the envelope from Peak 2 suggests a HC (Figure 4B).

Deconvolution of each of the peaks suggests that Peak 1 is attributed to a deamidated LC, while the Peak 2 could be attributed to deamidated HC (Figure 5). The HC and LC with no deamidation are co-migrating in Peak 3. The assignment of Peak 4 is unknown.

Analysis of smaller subunit fragments permits a higher confidence in N →G assignment.

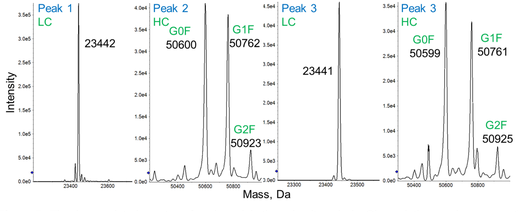
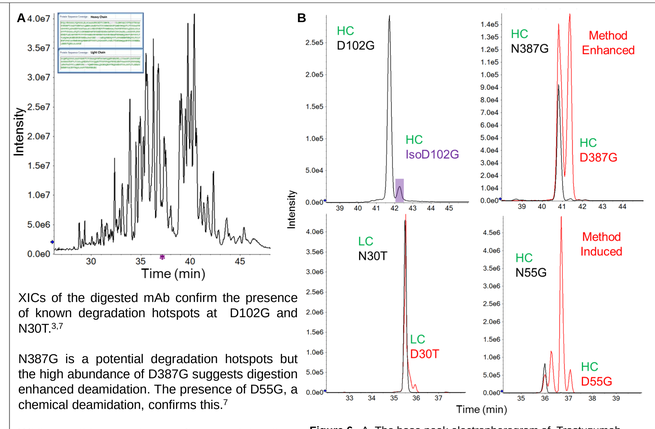


Figure 5. The deconvoluted spectra of each peak in the subunit analysis. Glycosylation can be seen on the HC.

3. Peptide analysis

Finally, CESI-MS peptide mapping can be employed to localize modifications and degradation hotspots to an amino acid residue.[5] Trastuzumab was reduced, alkylated and digested with trypsin (Figure 6A).

Tryptic peptide fragments typically range in size from 150 to 3000 Da. The CESI separation successfully separates both D → IsoD and N → D variants.[5,6] Further, high resolution TOF-MS instrumentation is highly suitable for the analysis of small (ΔM = + 1 Da) mass shifts. This assay confirms and localizes D isomerization and N deamidation. Unfortunately, the digestion process can not only artificially enhance deamidation but also create new "method induced" chemical deamidation events (Figure 6 B).[7]



XICs of the digested mAb confirm the presence of known degradation hotspots at D102G and N30T.[3,7]

N387G is a potential degradation hotspots but the high abundance of D387G suggests digestion enhanced deamidation. The presence of D55G, a chemical deamidation, confirms this.[7]

While peptide mapping confirms and localizes modifications the extensive sample preparation can prove detrimental and in some case intact analysis may be preferable.

Figure 6. A. The base peak electropherogram of Trastuzumab peptide mapping. B. The XICs of known degradation hotspots and known method induced degradation sites.

CONCLUSIONS

CESI-MS was employed to characterize the charge variants and potential CQAs of Trastuzumab. CESI-MS facilitates a multilevel mAb characterization using a single analytical platform. A single assay at the intact level enabled charge heterogeneity profiles, CQA identities and glycosylation profiles to be understood. CESI-MS further confirmed the localization of the potential CQAs to the subunit fragment as well as to a particular amino acid residue.

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